(a) a nucleotide sequence encoding an amino acid sequence represented by SEQ ID NO: 2 or 4 and having nicotianamine aminotransferase activity, or

(b) a nucleotide sequence which hybridizes to the nucleotide sequence of (a), when incubated in a solution of 5 x Denhart's solution, 5x SSPE and 0.1% SDS at 65°C for 12 hours, said nucleotide sequence encoding an amino acid sequence having nicotianamine aminotransferase activity.

REMARKS

Claims 2-11 and 13-20 are pending. No new matter has been added by way of the above amendments. For example, claim 2 has been amended to insert the phrase "said nucleotide sequence encoding protein having nicotianamine aminotransferase activity". As suggested by the Examiner, claim 11 has been amended to incorporate the subject matter of dependent Claim 12 as well as the subject matter supported by the present specification at page 10, lines 2-8 and page 18 common lines 8-9. Lastly, Claim 13 has been amended to be placed into independent format. Accordingly, no new matter has been added.

Applicants further submit that no new issues have been raised by way of the above amendments. For example, the amendments made to claim 2 and indirectly to claim 13 were suggested by the Examiner, thus, issues have been removed by way of these amendments. Claim 11 have been amended to include new

subject matter of a previously pending and considered claim, thus, no new issues have been raised by this amendment.

In view of the following remarks, applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims.

Non-Elected Claims

At page 2, subheading 4 of the outstanding Office Action the Examiner indicated that claims 14–20 drawn to an non-elected invention must be cancelled in order for a reply to the Final Office Action to be complete. Applicants respectfully disagree with the Examiner. There is no need to cancel the non-elected claims 14-20 at this time, since the Examiner has not indicated that all the other pending claims are in condition for allowance.

Objection to the Sequence Listing

At page 3, subheading 3 of the outstanding Office Action, the Examiner asserts that the Sequence Listing submitted on July 26, 1999, was received and entered. However, the Examiner asserts that some sequences were not assigned sequence identifiers. Applicants respectfully traverse. In particular it appears as though the amendments to the specification directed in the July 26, 1999 amendment were not properly entered. Specifically, by referring to page 3 of the July 26, 1999 Amendment it is apparent that correct sequence identifiers were added to, for example, page 25, of the specification. In

the letter regarding sequence listing requirements dated August 2, 2000, Applicants pointed out this discrepancy to the Examiner. Accordingly, this objection is overcome.

Issues Under 35 U.S.C.§ 112, First Paragraph

Claims 2 and 5-13 have been rejected under 35 U.S.C. 112, first paragraph, with the Office Action asserting that the recitation of hybridization conditions and part (b) of claim 2 in the absence of a functional activity fails to provide an adequate written description. Applicants respectfully traverse. In an effort to expedite prosecution, Applicants have amended claim 2 to insert the phrase "said nucleotide sequence encoding a protein having nicotianamine aminotransferase activity" as suggested by the Examiner. Accordingly the rejection is moot, and reconsideration and withdrawal thereof are respectfully requested.

The Examiner has also rejected claims 11-13 under 35 U.S.C 112, first paragraph asserting that the present specification fails to provide one of ordinary skilled in the art the enablement for enhancing the iron-absorbent ability of a host cell. Applicants respectfully traverse.

In the Examiner's rejection, the Examiner asserted that the present specification should provide more information. It is apparent that one of skill in the art, upon reading the present specification would not be presented with undue experimentation to practice the invention. The Examiner is therefore requested

to withdraw this rejection. In addition, Applicants are providing herewith a document entitled "Plant Biology '99 Program July 1999, Poster Sessions #427" in which the success of "Iron-deficiency-tolerant rice introduced with Nicotianamine aminotransferase of barley" was reported. This publication lends further credence to the above arguments for establishing that those skilled in the art are not presented with such an undue burden. Accordingly, the Examiner is requested to withdraw this rejection.

Allowable Subject Matter

At page 5, in the last paragraph of the outstanding Office Action, the Examiner states that claims 2-10 are free of the prior art. Additionally, the Examiner asserts that claim 3 and 4 are objected to as being dependent upon a rejected base claim. By overcoming the above rejections to claim 2, Applicants respectfully submit that claims 2-10 have now been placed into condition for allowance. Additionally, by overcoming the above rejections with respect to claims 11-13, claims 11-13 are also in condition for allowance.

If the Examiner has any questions or comments with respect to the above issues, please contact Craig A. McRobbie, Registration No. 42,874, at the offices of BIRCH, STEWART, KOLASCH & BIRCH, LLP.

Pursuant to the provisions of 37 C.F.R. § 1.17 and 1.136(a), the Applicant petitioned for an extension of three (3)

months to January 6, 2001 for the period in which to file a response to the outstanding Office Action dated July 6, 2000 in the concurrently filed Notice of Appeal. The required fee has been paid with the proper filing of this Notice of Appeal.

If necessary, the Commissioner is hereby authorized in this, concurrent, and further replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment

response to high concentration of sodium chloride and zinc chloride, the transcript level averaged 2–3-fold higher in 72 h. A similar trend in the induction of glyoxalase I protein and enzyme activity was seen. The leaf discs of transgenic plants over-expressing glyoxalase I showed significant tolerance to methylglyoxal, high salt and zinc. A comparison of plants expressing high and low level of glyoxalase I showed that the tolerance to salt and zinc concentrations was correlated with the degree of glyoxalase I expression. Our results suggest an important role of glyoxalase I in conferring tolerance to plants under stress conditions.

426 Session 41, Transgenics and Biotechnology

Ecpression of Arabidopsis CBF1 gene confers chilling tolerance in transgenic tomato. Chiu, Li-Hui Wu, Jing-Fen Charng, Yee-Yung Chan, Ming-Tsair *Institute of BioAgricutural Sciences, Acadeia Sinica, Nankang, Taipei, Taiwan* Wang, Yu-Chie *Department of Biology, National Taiwan Normal University, Taipei, Taiwan.* Presenter: Chan, Ming-Tsair mbmtchan@ccvax.sinica.edu.tw

Many tropical and subtropical crops are quite sensitive to low temperature, which usually result in chilling injury of plant tissues. Due to the susceptibility to chilling injury, the growing season of the crops is limited, and the quality of postharvest produce could threaten. This problem has drawn a lot of attention are reflected by large amount of literature on chilling injury. The Arabidopsis CBF1 cDNA driven by CaMV 35S and Barley Hva1 promoter, respectively, was transferred into tomato. The transgene integration was detected by Southern blot analysis and foreign expression was proven by Northern blot analysis, GUS assay and Western blot analysis. Overexpression of CBF1 cDNA in transgenic tomato induces strong expression of the target genes under untreated conditions. These transgenic tomatos show normal phenotype. These transgenic plants revealed freezing and dehydration stress conditions. These results indicate that the Arabidopsis CBF1 cDNA could function as a switch factor in transgenic tomato under low-temperature condition.

427 Session 41, Transgenics and Biotechnology

Iron-deficiency-tolerant rice introduced with Nicotianamine aminotransferase of barley. Michiko, Takahashi Hiromi, Nakanishi Takeo, Tanaka Naoko, Nishizawa K. Satoshi, Mori *Department of Applied Biological Chemistry, The University of Tokyo* Michiko, Takahashi Satoshi, Mori *Core Research for Evolutional Science and Technology* Shinji, Kawasaki *National institute of Agrobiological resources* Presenter: Michiko, Takahashi aa1078@hongo.ecc.u-tokyo.ac.jp

Nicotianamine aminotransferase (NAAT), the key enzyme involved in the biosynthesis of mugineic acid family phytosiderophores (MAs), catalyzes the amino transfer of nicotianamine (NA). MAs are found only in graminaceous plants. The amino transfer reaction catalyzed by NAAT is the first step in the unique biosynthesis of MAs. The amount of MAs secreted and NAAT activity increased under Fe-deficiency stress are correlated with of a plant's tolerance to Fe-deficiency. Of the graminaceous plants, barley is the most tolerant to Fe-deficiency and secretes the largest amount of MAs, while rice is the most susceptible to Fe-deficiency and secretes very few MAs. We purified NAAT proteins and isolated two genes (*naat-A* and *naat-B*) encoding NAAT from Fe-deficient barley roots. In addition, a 11.2 kb genomic fragment containing both *naat-B* and *naat-A* in this order was obtained and fully sequenced. We introduced CaMV35S-*naat-A* by using a pIG121Hm vector or the above mentioned genomic fragment containing both *naat-B* and *naat-A* using a pBIGRZ1 vector into rice (Oryzae sativa L. cv. Tsukinohikari). Several lines of transgenic rice highly expressing NAAT showed greater tolerance to iron deficiency in calcareous soil. It was confirmed that the development of cultivars tolerant to Fe-deficiency is possible by introducing the genes involved in biosynthesis of MAs. And this transgenic rice will contribute to solve the food problem and the environmental problem.

428 Session 41, Transgenics and Biotechnology

Synthesis of a protein-engineered heat-stable (1,3–1,4)-β-glucanase in maturing and germinating barley grains. Horvath, Henriette *Dept. of Crop and Soil Sciences & Genetics and Cell Biology, Washington State University* Huang, Jintai Wong, Oi T. von Wettstein, Diter Presenter: Horvath, Henriette henny@mail.wsu.edu

Barley plants expressing a bacterial hybrid gene encoding a heat-stable (1,3–1,4)-β-glucanase in the endosperm during grain maturation have been produced by *Agrobacterium*-mediated transformation. The binary vectors contain the *bar* gene for transformant selection, the green-fluorescent protein (GFP) gene as a screenable marker and the gene encoding the heat-stable β-glucanase. A high G+C (66%) and low G+C (47%) version of the bacterial hybrid gene is being tested for expression efficiency. The transgenes were provided with the D-hordein gene promoter and signal peptide code for targeting the enzyme into developing protein bodies of the endosperm. Western blots showed that transformants with the codon-optimized gene express large amounts of heat-stable enzyme during grain maturation, which remained active during seed germination. The amount of heat-stable (1,3–1,4)-β-glucanase was twofold higher than in our established lines expressing the recombinant β-glucanase under the control of the barley high-pI α-amylase gene promoter. The expression levels of the heat-stable enzyme in germinating grains of the lines tested in three years of field trials were faithfully inherited and ranged from 0.1–1.2 μg/mg soluble protein. Micro malting experiments of 17 homozygous transgenic lines showed a strong induction of heat-stable β-glucanase synthesis during germination, and transgenic seeds maintained greater than 80% of their activity during high temperature kilning. Interestingly the embryo of mature dry grains contained heat-stable β-glucanase, implying that the α-amylase promoter is leaky. The experiments demonstrate the feasibility of producing value adding recombinant proteins in the barley grain.

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Responses of transgenic plants overexpressing antioxidant enzymes to bacterial and viral infection. Ajit, Seena K. Zilinskas, Barbara A. *Plant Science Department, Rutgers University* Kobayashi, Donald Y. Hillman, Bradley *Plant Pathology Department, Rutgers University* Presenter: Ajit, Seena K. seena@eden.rutgers.edu

The production of active oxygen species probably plays a key role in plant defense against pathogens. It is the first identifiable response in many incompatible interactions, and in turn initiates the hypersensitive response (HR). Transgenic Samsun NN tobacco plants were constructed which express ascorbate peroxidase (APX) or Cu/Zn superoxide dismutase (SOD) in the apoplast or cytosol; the former scavenges H_2O_2, and the latter produces H_2O_2 and removes superoxide. These plants were tested for their response to bacterial and viral pathogens. Plants were inoculated with *Pseudomonas syringae* pv *tabaci*, which is pathogenic to tobacco, or *P. syringae* pv *lacrymans*, which causes an HR. Bacterial growth was monitored 1, 24, 96 and 168 hrs after inoculation. There was no statistically significant difference in bacterial growth in either APX or SOD (apoplastic and cytosolic) overexpressors compared to the control plants. These plants were also tested for their resistance response to TMV. Both the apoplastic and cytosolic overexpressors of SOD showed enhanced resistance as demonstrated by the two- to three-fold decrease in the number of HR lesions 48 hrs after infection compared to the control plants. To further test if overexpression of SOD could confer protection in the absence of the *N* gene, the TMV-sensitive cultivar of tobacco, Bel W3, was infected with the virus. Two weeks after infection, leaves at different stages of development were analyzed by western blots for TMV coat protein. There was no difference in TMV multiplication in either the APX or SOD transgenic plants relative to the control. Thus, these results suggest that having increased quantities of H_2O_2 can endow increased protection against virus infection only in the presence of the *N* gene.

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Oxidative stress management-targeting MnSOD to the chloroplast. Davuluri, Ganga Rao *Avesthagen Graine, Plant Genome Biology Laboratory, Basic Sciences Building, University of Agricultural Sciences, GKVK Cempus, Bangalore-560065* Chettoor Mathai, Antony Nirmal, Rashmi Azhagiri, Arun Kumar Morawala Patell, Villoo Presenter: Chettoor Mathai, Antony cmantsy@yahoo.com

In eukaryotes, MnSOD is a nuclear-encoded protein, that scavenges superoxide radicals in the mitochondrial matrix. By targeting this enzyme to the chloroplast, where the generation of superoxide radicals is high during stress conditions, the capacity to scavenge any radical that may be produced can be increased. Increased production of superoxide radicals is associated with a number of physiological disorders in plants. We have generated transgenic rice plants by co-transforming three week old scutellum callus using particle accelerator Biolistic PDS-1000/He with plasmids pGV2 and pILTAB222. The plasmid pGV2 carried the MnSOD cDNA cloned downstream of CvMV promoter and the chloroplast targeting peptide followed by the nos terminator. The pILTAB222 carried the hygromycin B phosphotransferase downstream of 35S CaMV promoter and followed by the nos terminator. We have proved the presence of the gene in both To and T1 generations by PCR, Southern and Northern analysis.The production of the native engineered protein has been assayed and immunolocalized to the chloroplast. We will present molecular, biochemical and physiological data for identification of the expressing plants for field trials and product development.

431 Session 41, Transgenics and Biotechnology

Analysis of production and activity of an *Arthrobotrys oligospora* proteolytic enzyme in tobacco and bacteria. Ponce, Liana P. Potenza, Carol Thomas, Stephen Sengupta-Gopalan, Champa *New Mexico State University* Presenter: Ponce, Liana P. liponce@nmsu.edu

Plant-parasitic nematodes represent a primary class of plant pathogens with enormous worldwide impact. Each year, crop damage caused by phytopathogenic nematodes totals billions of dollars. One approach to building nematode resistance in plants has been to exploit the natural mechanisms used by various